

AMENDMENTS TO AND LISTING OF THE CLAIMS

This listing of the claims will replace all prior versions and listings of the claims in this application.

Please amend the claims as follows:

1. (Previously Presented) A method for coamplification of two or more target nucleic acids having different sequence compositions present at comparable copy numbers wherein the maximum difference between the lowest and highest copy number is 10-fold said method comprising at least 15 primary amplification cycles, each amplification cycle comprising the sequently steps of:

(A) heating a reaction mixture of two or more target nucleic acids, or their primer extension products, at a first temperature, T_1 , for denaturation of the strands of the target nucleic acids or their primer extension products, and

(B) priming the denatured strands with a set of primers specific to and hybridizable with opposing strands of each target nucleic acid to be amplified, by cooling to a second temperature, T_2 , and

(C) either as a continuation of step (B) or in a separate step, forming primer extension products in a reaction mixture of PCR reagents, by incubation at a third temperature, T_3 , provided that when priming and primer extension product formation are carried out in the same step, T_2 and T_3 are the same, and wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 20 weight % of a nonionic, polymeric volume exclusion agent, a thermostable hot start DNA polymerase, and optionally a sequence specific labeled probe which binds with the primer binding regions and which is detectable after hybridization, and

(D) in the course of the reaction in each amplification cycle or in an amplification cycle after the last primary amplification cycle detecting one or more of the primer extension products as an indication of one or more of the target nucleic acids.

2. (Previously Presented) The method for coamplification of two or more target nucleic acids having different sequence compositions according to claim 1, wherein the amount of nonionic polymeric volume exclusion agent in said reaction mixture is 1 to 15 weight %.

3. (Canceled).

4. (Previously Presented) The method for coamplification of two or more target nucleic acids having different sequence compositions according to claim 1, wherein the amount of nonionic polymeric volume exclusion agent in said reaction mixture is 1 to 8 weight %.

5. (Previously Presented) The method according to one of claims 1, 2, or 4, characterized in that the volume exclusion agent is selected from the group consisting of a polyether, a reaction product of a sugar with epichlorohydrin, a polysaccharide, and a polyacrylate.

6. (Previously Presented) The method according to claim 5, characterized in that the volume exclusion agent is selected from the group of polyethers of the general formula:



wherein R is an alkylene bridge of 1 to 6 carbon atoms - branched or unbranched - and n is an integer of 15 to 1000.

7. (Previously Presented) The method according to claim 6, characterized in that R may represent 1,2-ethylene, 1,3-propylene, 1,2-propylene, 2-hydroxy-1,3-propylene, 3-hydroxy-1,2-propylene, 1,4-butylen, 1,3-butylen, or 1,2-hexylene.

8. (Previously Presented) The method according to claim 6, characterized in that the polyether is poly(ethylene glycol).

9. (Previously Presented) The method according to claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 1000 to 2,000,000 daltons.

10. (Previously Presented) The method according to claim 8, characterized in that the poly(ethylene glycol) has a molecular weight in the range of 3000 to 500,000 daltons.

11. (Previously Presented) The method according to claim 8, characterized in that the poly(ethylene glycol) has a molecular weight of about 8000 daltons.

12. (Previously Presented) The method according to claim 5, characterized in that the volume exclusion reagent is a dextran.

13. (Previously Presented) The method according to claim 12, characterized in that the dextran has a molecular weight in the range of 1000 to 2,000,000 daltons.

14. (Previously Presented) The method according to claim 12, characterized in that the dextran has a molecular weight in the range of 3000 to 500,000 daltons.

15. (Previously Presented) The method according to claim 12, characterized in that the dextran has a molecular weight in the range of 40,000 to 60,000 daltons.

16. (Previously Presented) The method according to claim 5, characterized in that the polyacrylate is selected from the group consisting of poly(hydroxyethyl acrylate) or poly(2,3-dihydroxypropyl acrylate).

17. (Withdrawn) An amplification reaction composition which is buffered to a pH of from about 7.5 to about 9, and wherein said composition comprises a mixture of the following:
one or more sets of primers,
a thermostable hot-start-DNA polymerase,
a plurality of dNTP's, and
1 to 20 weight % of a nonionic, polymeric volume exclusion agent, and

optionally a sequence specific labeled probe which binds within the primer binding regions and which is detectable after hybridization.

18. (Withdrawn) The amplification reaction composition of claim 17, wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 15 weight % of a nonionic, polymeric volume exclusion agent.

19. (Withdrawn) The amplification reaction composition of claim 17, wherein the reaction mixture in at least one of the primary amplification cycles comprises from 1 to 8 weight % of a nonionic, polymeric volume exclusion agent.

20. (Withdrawn) A kit for the coamplification of two or more target nucleic acids according to the method of Claim 1, comprising:

(a) an amplification reaction composition buffered to a pH of from about 7.5 to about 9 and comprising:

one or more sets of primers,
a thermostable hot-start DNA polymerase,
a plurality of dNTP's, and
1 to 20 weight %, preferably 1 to 15 weight %, most preferably 1 to 8 weight %, of a nonionic, polymeric volume exclusion agent, and

(b) a capture reagent comprising an oligonucleotide immobilized on a water-insoluble substrate.

21. (Withdrawn) A self-contained test device for performing the amplification method of Claim 1, comprising, in separate compartments:

(a) an amplification reaction composition buffered to a pH of from about 7.5 to about 9 and comprising:

one or more sets of primers,
a thermostable hot-start-DNA polymerase,
a plurality of dNTP's, and

1 to 20 weight %, preferably 1 to 15 weight %, most preferably 1 to 8 weight %, of a nonionic, polymeric volume exclusion agent, and,

(b) a capture reagent comprising an oligonucleotide immobilized on a water-insoluble substrate, the compartments being connected in the test device so that the amplification reaction composition can be brought into contact with the capture reagent after amplification without opening the test device.

22. (Withdrawn) A kit for preparing an amplification reaction composition according to claim 17 comprising:

- at least one hot-start DNA polymerase, and
- at least one polymeric exclusion reagent.

23-24. (Canceled).

25. (Previously Presented) The method according to any one of claims 1, 2, or 4 wherein said chemically thermostable hot start DNA polymerase is modified by reaction with an aldehyde.

26. (Previously Presented) The method according to claim 1, characterized in that one of the primers of each primer set is fluorescently labeled.

27. (Previously Presented) The method according to claim 1, characterized in that one of the primers of each primer set is labeled with a specific binding moiety.

28. (Previously Presented) The method according to claim 1, characterized in that the sequence specific labeled probe is fluorescently labeled.

29. (New) The method according to claim 1 wherein each set of primers hybridizable with opposing strands of each target nucleic acid to be amplified is different.

30. (New) The method according to claim 1 wherein the method is capable coamplifying six (6) different target nucleic acids.
31. (New) The method according to claim 1 wherein the method is capable coamplifying eight (8) different target nucleic acids.
32. (New) The method according to claim 1 comprising six (6) different target nucleic acids.
33. (New) The method according to claim 1 comprising eight (8) different target nucleic acids.
34. (New) The method according to claim comprising eight (8) sets of primers.